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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Tai-Shun Lin and William H. Prusoff

Serial No. : 06/942,666

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For : USE OF 3'-DEOXYTHYMIDIN-2'-ENE (3'-DEOXY-2', 3'-DIDEHYDROTHYMIDINE) IN TREATING PATIENTS INFECTED WITH RETROVIRUSES

Art Unit : 183

Examiner : Dr. John W. Rollins

Hon. Commissioner of Patents
& Trademarks
Washington, D.C. 20231

Sir:

DECLARATION UNDER RULE 132

Lisa M. Dunkle declares that:

1. All statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

4. Phase I clinical studies of d4T were begun in 1989 and have involved 54 subjects to date. All subjects were infected with the Human Immunodeficiency Virus and exhibited evidence of that infection including deficient numbers of CD4 antigen-bearing lymphocyte counts of clinical symptoms of AIDS or AIDS-related complex, including weight loss, opportunistic infections, diminished performance scores and, in some, presence of detectable HIV p24 antigen in the blood. The purpose of these studies was to evaluate the pharmacokinetics (degree of absorption from the intestinal tract, blood levels of d4T and rates of elimination from the body) of several escalating doses of d4T and to assess evidence of clinical efficacy. In addition, careful assessments were conducted to assess any toxic effects which might have resulted from d4T administered in a variety of doses. Clinical efficacy was determined by diminution in the symptoms of HIV infection listed above, as well as improvement in the laboratory parameters of HIV infection, i.e., improvements in CD4 cell counts and falls in p24 antigen levels. Toxic effects were sought in clinical adverse reactions as well as laboratory evidence of bone marrow, liver or kidney damage. Results of an interim analysis of these data are now available.

5. <u>CD4 Cell Counts</u>: Data analysed from the first 33 patients followed for a median of 12.3 weeks, and as long as 6 months, indicated sustained elevations in CD4 cell counts from baseline in 11 of 31 subjects who received doses

of d4T ranging from 2 - 12 mg/kg/day orally. The CD4 cell count has been shown to be a sensitive indicator of the degree of immunosuppression due to HIV infection and falls in CD4 count are the most reliable laboratory indicator of adverse progression of HIV infection. Rises in CD4 cell counts significantly above the initial value or to a count in excess of 300/cu mm are interpreted to indicate an improvement in the immune system resulting from antiviral effect of d4T.

6. HIV p24 antigen: Of the 11 patients who had detectable blood levels of p24 antigen prior to d4T treatment, 10 exhibited sustained falls of at least 50% or to undetectable levels. Elimination of detectable p24 is interpreted as laboratory evidence of antiviral efficacy because the quantity of antigen in the blood reflects the quantity of replicating HIV virus in the blood. Patients who do not exhibit detectable p24 antigen prior to antiretroviral therapy are those whose viral antigen is bound to anti-p24 antibody, and techniques for measuring anti-retroviral activity in those patients are not yet established. The observation that p24 antigen is eliminated in most patients receiving d4T reflects remarkable anti-HIV activity.

7. Clinical Observations: Because the intent of a Phase I study is to evaluate a range of doses of a drug to determine the relative safety of the various doses, dose-related toxicities are intentionally sought. Such toxicities were observed in patients receiving doses of d4T

above 2 mg/kg/day in 4 divided daily doses and doses above 4 mg/kg/day in three divided daily doses. The maximum tolerated dose was determined to be 2 mg/kg/day divided qid and 4 mg/ky/day divided tid. The dose-limiting toxicities were painful peripheral neuropathy and liver enzyme elevation suggestive of potential liver damage. Anemia was encountered at the highest doses of 8 and 12 mg/kg/day. Nausea, diarrhea, isomnia and dizziness occurred occasionally, but did not interfere with dosing at the 2 and 4 mg/kg/day levels.

Clinical improvement in the parameters of HIV infection was observed in most patients. Twenty of 30 patients exhibited improvement in symptom/performance scores and 11 of 31 patients had sustained weight gain of greater than 2.5 kg. At the dose levels of 2 and 4 mg/kg day (the latter divided tid), improvements in the two clinical parameters listed above were seen in 5 of 9 and 9 of 13 patients respectively.

8. Combined Clinical/Laboratory Responses: Improvement in CD4 and/or p24 antigen levels was seen in 17 of 31 patients at all dose levels and in 10 of the 13 patients at the two lower doses. Improvements in either or both clinical paramenters were noted in 24 of 33 patients at all dose levels and in 9 of the patients at the two lower doses. Improvements in these clinical manifestations of HIV infections presumably reflect amelioration of the infection and efficacy of d4T.

9. Data demonstrating the anti-HIV effectiveness of ddC, d4T and AZT in vitro in various cell culture systems which are available in the files of the Bristol-Myers Squibb Company are summarized in the following table.

In Vitro Anti-HIV

Compound	Assay/Cell	Id_{50} (μm)[1]	TD_{50} (μm)[2]
ddC[3]	viability/ATH-8	0.1	32
	p24 antigen/CEM	<0.001	<0.1
d4T	p24 antigen/CEM-F	0.15	
AZT[4]	p24 antigen/CEM	0.45	54
	viability/ATH-8	6.0	
	p24 antigen/CEM-F	0.1	

[1] Concentration producing 50% inhibition of viral replication

[2] Concentration producing 50% inhibition of cell viability

[3] ddC: 2',3'-dideoxycytidine

[4] AZT: 3'-azido-3'-deoxythymidine

Date 5/22/90

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